Development of a simulation tool based on a segregated model to optimize the design and the scale up of animal cell culture in fixed-bed bioreactor

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The fixed-bed bioreactor is a promising system for the process intensification of the adherent animal cell culture. Nevertheless the fixed-bed bioreactor presents heterogeneity of the cell and the species concentrations which can complicate its optimization and its scale-up. The aim of this work is to develop a mathematical model of the evolution of the cell concentration and the species concentrations to study the process optimization and the bioreactor scale-up. The developed model is used as a simulation tool to study the influence of different phenomena on the cell heterogeneity. In this work, the importance of the adherent phase is investigated. This phase takes place in the beginning of the process. To realize a good implementation of the process, it is important to control the adherent cell concentration and to minimize the heterogeneity during this phase. If cell concentration heterogeneity appears, it will have repercussions during the whole process. In the model, four cell populations are considered: the viable cells in suspension in the medium, the captured cells by the fixed-bed in suspension in the medium, the adherent cells on the fixed-bed and the dead cells in suspension in the medium. Five extracellular species are considered: glucose, glutamine, oxygen, ammonia and lactate. Five phenomena are modeled: the culture medium flow through the fixed-bed (with axial convection, radial dispersion and axial dispersion), the cell capture by the fixed-bed, the cell adherence on the fixed-bed, the cell growth with a maximal cell concentration imposed by the specific area of the fixed-bed and the cell death. The interaction between cells and species is modeled by a Monod equation for the specific growth rate. The model equations are solved with a routine developed with Matlab 6.5. This routine used the Finite Volume Method coupled with a Newton-Raphson algorithm. The model parameters are experimentally identified by cell cultures in a pilot bioreactor. In this bioreactor, cultures are also realized to validate the model. The influence of cell capture is investigated. Simulations are realized with different values of the kinetic constant of cell capture. In the simulations, the cell concentration gradients are larger if the kinetic constant is larger. The kinetic constant experimentally identified is proportional to the carrier concentration in the fixed-bed. Thus the carrier concentration has to be chosen with precaution. The influence of axial velocity is also studied. To increase the homogeneity factor of the cell concentration during the adherence phase, the axial velocity can be increased. The velocity field inside the fixed-bed has also an influence on the homogeneity of the cell concentration. Thus the modification of the inlet velocity profile is an interesting solution to increase the homogeneity of the fixed-bed. A flat inlet velocity profile should be preferred. Therefore the design of the grid which maintains the carrier in the fixed-bed can be modified to obtain an inlet velocity profile more interesting. For the design of the bioreactor, a radial scale-up should be preferred to an axial scale-up.

Keywords. Fixed-bed bioreactor, segregated model, adherent phase.

Metabolic flux analysis of an underdetermined network of CHO cells

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The flux distribution in a detailed metabolic network of CHO-320 cells is evaluated using Metabolic Flux Analysis. As in many practical situations, the available information are not sufficient to completely define the metabolic fluxes, and so, the mass balance system of equations is underdetermined. However, the measurements of the time evolution of a number of extracellular components can provide a set of constraints on the metabolic network, so that a range of possible (non-negative) solutions for each metabolic reaction can be computed instead. In this way, metabolic flux intervals can be established for each intra-extracellular flux in the metabolic network. Moreover, the incorporation of simple theoretical assumptions or the addition of further extracellular measurements, result in the determination of certain metabolic fluxes and the delimitation of quite narrow intervals for the others, so providing a good guess of the real flux distribution in CHO-320 cells. An unique flux distribution can also be computed through linear optimization and the definition of some optimality criteria. In addition, the chosen network structure is analyzed in the light of the different possible configurations that the metabolic network can take due to the alternation of some of their reversible reactions.

**Keywords.** Mammalian cells, metabolic flux analysis, flux optimization.